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Antibiotic-loaded nonwovens as protection against implant-associated infection

Abstract: Implant-associated infections still must be considered as a major risk factor and represent a significant threat to the well-being of patients. The detection of such complications is often late, making therapy at this stage difficult. However, the common route of systemic antibiotic prophylaxis can be associated with numerous side effects on the patient or even lead to resistant strains of bacteria. In this context, local antibiotic drug load is a promising way to protect implants against pathogen adhesion and biofilm formation. This work addresses the incorporation of the antibiotic Doxycycline into a biodegradable nonwoven matrix as potential implant coating. While the nonwoven matrix itself displayed already inhibitory effects on *Staphylococcus aureus* (*S. aureus*) biofilm formation in vitro, this effect was even more pronounced by the incorporation of Doxycycline.

Antibiotic loaded nonwovens display the possibility for local inhibition of biofilm formation. In the shape of an implant coating, this may further help to avoid implant-associated infections. Nevertheless, as pathogens vary in shape and type, further adjustments have to be performed to improve wide-ranging protective effects

Keywords: Nonwoven, antibiotic, Doxycycline, PCL; drug delivery

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1 Introduction

Implants are a cutting-edge technology in contemporary medicine and substantially improve the medical prognosis and patients' quality of life. However, as versatile as the areas of implant applications are, there are several complications, with peri-implant infection (PII) being one of the major ones. A prominent example is PII of osteosynthesis implants, in which context, *S. aureus* being the most common representative of biofilm-forming virulent microorganisms [1]. Still, due to a broad range of accessible antibiotics for systemic application, the incidence of PII is relatively low (1-5%) compared to the increasing number of osteosynthesis implant insertion [2]. However, with the risk of developing a manifested sepsis and thus propagation to internal organs, leading to consequential complications, PII remain a severe and serious concern.

Furthermore, PII plays a considerable role concerning cardiac pacemaker (PM) and defibrillator implants [3]. The risk of postoperative PM aggregate infection is reported to be 0.5 - 1% and even up to 1.7% for biventricular PM aggregates, respectively, for the first six to twelve months and increases to 9.5% after two years, representing a serious clinical problem [4]. The most frequent viral microorganisms associated with PM infections are Methicillin-resistant *S. aureus* (MRSA) (23.4%) and Methicillin-sensitive *S. aureus* (14.9%), *Pseudomonas* (10.6%), *E. coli* (8.5%), and *Klebsiella* (6.4%) [5].

PM aggregate infections are often diagnosed late and are potentially fatal. Treatment of the infection is in the majority of cases associated with a change of the pacemaker aggregate or even the complete removal of the system including the electrodes and represents an enormous burden for the patient and the health care system [6]. The clinical consequences of a manifested PM infection range from local tissue damage to sepsis and include symptoms such as localized pain, redness (erythema) and heating, swelling, purulent discharge, and eversion (ulceration) of the generator or electrodes through the skin [7].

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In general, the two main goals of PII prophylaxis are 1: Preventing bacterial adhesion and biofilm formation and 2: Inhibition of the promotion of pathogen resistance. In this context, the generation of antibacterial implant surfaces is an efficacious approach [8]. In the case of PM, immediate preoperative immersion of the aggregate in an antimicrobial solution considerably reduces bacterial adhesion and the risk of early PII [9]. Thus, application of controlled release systems with release periods from days to weeks paired with antibacterial implant surfaces may drastically decrease PII case rates in the near future [8,10].

Within this work, we present an antibiotic nonwoven material as potential antibacterial implant covering for the prevention of PII. Electrospinning is a versatile process to obtain fibrous materials in a high scalable process with good reproducibility and allows for the use of a broad palette of polymers and additives, in particular drugs [11–14]. By adding Doxycycline into the polymer solution used for the spinning process, we were able to incorporate the drug into the poly-ε-caprolactone (PCL) fibers. PCL has been chosen as model drug carrier due to its widespread use in biomedical engineering as biodegradable polymer. The drug did not show to influence the fiber morphology of the yielded material in comparison to the pure nonwoven. Initial experiments regarding drug release rates as well as the biofilm inhibitory potential of the material have been conducted. In summary, antibiotic nonwoven may lead to adaptable implant coverings and thus contribute in the reduction of severe PII.

2 Materials and Methods

2.1 General Information

2.1.1 Electrospinning of antibiotic loaded nonwovens

Electrospinning was performed on a Contipro 4SPIN C4S LAB2 (Dolní Dobrouč, Czech Republic). The setup used was 21 cm collector distance, 36 kV high voltage and a feed rate of 45 µL/min. Poly-ε-caprolactone (PCL) was dissolved in CHCl₃/MeOH with 7:1 ratio (PCL: 12 w/w% and Doxycycline (Sigma, Germany) 20 w/w% with respect to polymer mass) prior to spinning. The obtained nonwovens were thermally annealed for 24 h at 80 °C under reduced pressure to remove residual solvent.

2.1.2 Doxycycline release study

Drug release has been performed *in vitro* using 2 mL PBS (Morphisto, Germany) at pH = 7.4 at 37 °C with n = 3. For HPLC measurements, medium has been changed completely after 15, 30, 60, 120, 240 and 1440 min. Doxycycline concentration of the media was analysed using HPLC (KNAUER Wissenschaftliche Geräte GmbH, Germany) equipped with a 250x4 mm Eurosper-100 C18 5 µm column. The eluent was 0.1% trifluoroacetic acid (TFA) in H₂O and 0.085% TFA in acetonitrile with a ratio of 63:37. Measurements have been performed under isocratic conditions for 9 min and with the oven temperature set to 25 °C. Retention time of Doxycycline was $t_R = 4.1$ min.

2.1.3 Bacteria culture conditions

S. aureus (ATCC5556) and *E. faecalis* (ATCC 29212) were purchased from the American Type Culture Collection. *S. aureus* was cultured in LB-broth supplemented with 1% glucose. *E. faecalis* was cultured in BHI-Broth (Brain Heart Infusion Broth) supplemented with 1% glucose. The bacterial cultures were grown aerobically at 37 °C in 24-well polystyrene plates with a culture volume of 2 ml (Nunc, Thermofisher scientific, Germany). The inoculum of each strain was prepared by adjusting the concentration of an overnight bacterial broth culture to 1 x 10⁸ CFUs/ml in BHI or LB.

2.1.4 Quantification of biofilms by crystal violet assay

The biofilm formation was analyzed using crystal violet staining method. The inoculum of each strain was prepared by adjusting the concentration of an overnight bacterial broth culture to 1 x 10⁸ CFUs/ml in BHI or LB. Two milliliters of the suspension was added to 24-well plate, which contained disks of the different materials and was incubated at 37 °C for 24 and 72 h. At each time point, the supernatant was removed and the disks were washed three times with double-distilled water to remove non- or loosely adherent bacteria. Then for the purpose of heat setting the plates were incubated at 60 °C for 1 h. After that 200 µl of 0.06% crystal violet were given into each well and incubated 5 min. The crystal violet was removed and the disks were washed three times with double-distilled water. The elution of the biofilm from the disks was performed with 200 µl of 30% acetic acid. The supernatant was measured in a multilabel microtiter plate (FLUOstar Omega, BMG Labtech, Germany) at 600 nm. For values over 2.5 the supernatants have been diluted with 30% acetic acid. For the blank, 30% acetic acid was used.

2.1.5 SEM imaging

SEM imaging was performed using a SEM QUANTA FEG 250 (FEI Company, Germany) with an accelerating voltage of 10 kV and a proprietary spot size value of 3.0. The samples were fixed onto aluminum trays with conductive tape and sputter coated with gold at 0.06 mbar/25 mA for 2x120 sec.

3 Results

SEM imaging showed that electrospinning of PCL/Doxycycline lead to the formation of homogeneous fibers. No such things as beading or electrospraying, which would lead to polymer film formation, could be observed. However, fiber diameter appears to be considerable smaller with the incorporated drug.

Figure 1: SEM images of PCL nonwoven (A) and Doxycycline loaded PCL nonwoven (B).

In vitro drug release experiments from the Doxycycline loaded PCL nonwovens showed a high initial burst release during the first 60 min of the release experiment. This is in accordance with the anticipation, as the nonwoven has an extremely high surface-to-volume ratio. After 240 minutes, 100 $\mu\text{g}/\text{mg}_{\text{polymer}}$ were released. Thereafter, the shape of the Doxycycline concentration curve led to the formation of a plateau. Thus, we assume that there is still drug entrapped into the polymer fibers as 20 w/w% drug with respect to the polymer mass have been initially deployed. The release curve can be found in Figure 2.

Figure 2: Release curve for Doxycycline from PCL nonwoven in PBS at 37 °C and pH = 7.4.

Figure 3: Biofilm formation data for PCL nonwoven and PCL with incorporated Doxycycline. All data are given in respect to pure PCL nonwoven as reference.

Notably, biofilm formation experiments revealed that already pure PCL nonwoven showed a decrease in *S. aureus* biofilm formation after 24 h in comparison to polystyrene. This effect could be even enhanced by the incorporation of Doxycycline (Figure 3). However, due to release of the

antibiotic drug into the culture medium, an increase in biofilm formation could be observed after 72 h.

Regarding *E. faecalis*, also decreased biofilm formation was observed for PCL nonwoven compared to polystyrene. However, no further inhibitory effect of the incorporated drug has been found. Notably, after 72 h, a higher biofilm formation was observed for the PCL nonwoven with incorporated Doxycyclin compared to pure PCL.

4 Conclusion

Drug loaded nonwovens offer the potential for usage as antibiotic implant coating. Due to the adaptable shape and the inherent strain reserve, the material may be applied on a broad variety of implants such as cardiac pacemaker or defibrillator aggregates, neuro stimulator aggregates, cochlea implants or osteosynthesis implants. Due to the porous nature of the nonwoven, fast drug release can be achieved to ensure an early stage pharmacologically active drug level in the surrounding tissue or fluids. PCL nonwoven surface showed biofilm inhibitory effect itself for *S. aureus* and *E. faecalis*. In the case of *S. aureus*, this may be further enhanced by the addition of Doxycyclin.

Author Statement

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